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TRANSACTIONS

Studies on Vitamin B_2 Complex.

VII.—The Rat Growth Factor in Liver Filtrate, with Evidence for its Multiple Nature.*

By Ume TANGE and Hide SASAKI.

(Received October 23, 1940.)

In a previous paper,[1] one of us (U.T.) reported that the rat growth-promoting factor (or factors) in liver filtrate showed great resemblance in properties to factor W of Frost and Elvehjem[2].

Recently Jukes[3] and Wooley *et al.*[4] have demonstrated that the "chick antidermatitis factor" is probably identical with pantothenic acid, which has been investigated by Williams and co-workers[5]. But Hoffer and Reichstein,[6] and Subbarow and Hitchings[7] have also indicated that the rat filtrate factor is probably pantothenic acid. Edgar *et al.*[8] have reported that their liver filtrate factor has at least three components.

Thus it has been shown that the so-called "filtrate factor" contains several components which are required for normal growth of rats.

This paper is concerned with the study of the rat growth-promoting substance contained in the different highly purified fractions obtained from liver filtrate.

EXPERIMENTAL.

Since we have found that sucrose is a better constituent for basal rations in the study of vitamin B_2 complex than are starches which contain some of the factors, the basal ration employed in the present experiments is changed in composition from that previously used, as follows:

Purified fish protein	20%
Sucrose (pharmacopeia Japonica)	70%
Butter fat	5
Agar-agar	1
McCollum's salt mixture	4

* This paper was presented at the Scientific Meeting of I. P. C. R., June 14, 1940.

Young rats weighing between 40 and 50 g were placed on the basal diet supplemented daily with 20 γ of vitamin B_1 (synthetic B_1 chlor-hydrochloride), 30 γ of flavin,* 1 mg of nicotinic acid, and 2 drops weekly of biosterin as vitamins A and D. At the end of 3 weeks they were given daily, in addition to the above supplements, 20 γ of crystalline vitamin B_6 and an adequate amount of various liver fractions under investigation. The growth rate of the animals was observed for periods of at least six weeks.

Methods.

Preparation of liver extract fractions.

3 liters (1 cc=10 g of fresh beef liver) of methanol extract[1] of raw liver, which has an acid reaction and is of a pale green-yellowish colour, was treated twice with 200 g portions of acid clay to remove flavin and inert substances which might interfere with further purification of the filtrate. The filtrate from acid clay adsorbates was neutralized with NaOH, and concentrated to viscous consistency in vacuo; then methanol was added until no further precipitate occurred. After standing several hours to allow the precipitate to settle, the clear solution was decanted, and the solvent was removed by distillation under reduced pressure. This methanol-soluble fraction was used as the starting material for all the experiments. A portion of this material was kept for assay. The feeding results are shown in Fig. 2 (a). The remaining portion was further evaporated under reduced pressure to syrupy consistency, and then repeatedly extracted with acetone containing 10% of water. The acetone extract was distilled under reduced pressure and water added to make 1 cc equal to 100 g of fresh liver (fraction A). Feeding the material at the level corresponding to 10 g of fresh liver brought about normal growth (Fig. 2 (b)).

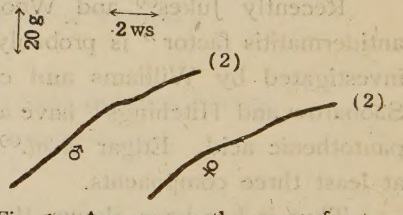

Fig. 1. Average growth curves of rats on diet without liver filtrate, control ration. The figures in parentheses indicate the number of rats in the experiments.

From the results shown in Fig. 2 (a) and (b), it appears evident that the active principle in methanol-soluble fraction is almost completely extracted with aqueous acetone.

The procedure employed for the concentration of the active substances is illustrated in Chart I.

The results shown in Fig. 3 (a) and (b), indicate that charcoal eluate (fraction B) contains about one-half of the original activity, whereas charcoal filtrate, about one-fourth.

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* Flavin was prepared from dried egg white powder by the method described in a previous paper (Sc. Pap. I. P. C, R., 35 (1938), 59).

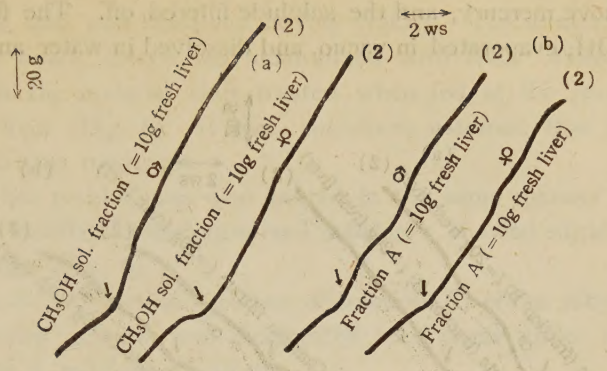

Fig. 2. Average growth curves of rats on control ration, supplemented with CH_3OH-soluble fraction (a) and with fraction A (b).

The figures in parentheses indicate the number of rats in the experiments.

Chart I.

Steps in the concentration of the growth-promoting substances in liver extract.

CH_3OH-soluble fraction (Fig. 2 (a)). Concentrate. Extract with acetone.

↓

Residue (inactive)	Acetone extract. Evaporate in vacuo, and take up in H_2O
H_2O insoluble residue (inactive)	Aqueous solution (fraction A, Fig. 2 (b)). Adjust to pH 2~3 with H_2SO_4. Treat six successive times with charcoal
Charcoal filtrate (active, Fig. 3 (b))	Charcoal adsorbate. Elute with 90% ethanol containing 0.1% pyridine
Eluted charcoal (inactive)	Alcoholic eluate. Evaporate in vacuo. Dissolve in H_2O, and add phosphotungstic acid in 1% H_2SO_4
Precipitate (inactive)	Phosphotungstic acid filtrate. Neutralize with saturated Ba(OH)
Ba phosphotungstate (inactive)	Filtrate (fraction B, Fig. 3 (a))

During the course of the procedure some attempts were made to ascertain further properties of the "rat filtrate factor":

Mercury precipitation.— 50 cc of fraction A was diluted with water and a solution of about 20% mercury acetate added until no further precipitate occurred. After standing overnight, the precipitate was centrifuged, the filtrate was treated

with H_2S to remove mercury, and the sulphide filtered off. The filtrate was neutralized with NaOH, evaporated in vacuo, and dissolved in water and tested on rats.

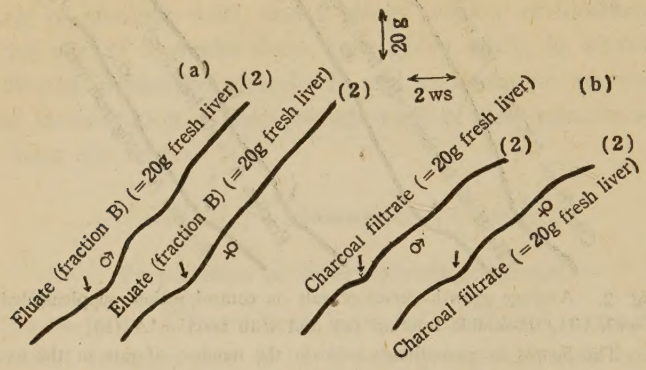

Fig. 3. Average growth curves of rats on control ration, supplemented with charcoal eluate (fraction B) (a), and with charcoal filtrate (b). The figures in parentheses indicate the number of rats in the experiments.

The mercury precipitate was suspended in water acidified with H_2SO_4, and Hg was removed by means of H_2S. The remaining material was treated as above and assayed on rats.

The mercury filtrate was found to have a growth-promoting activity approximately equal to that of fraction A, while the precipitate appeared inactive (Fig. 4).

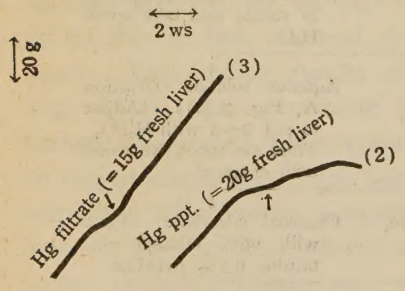

Fig. 4. Average growth curves of rats on control ration, supplemented with mercury filtrate or with the precipitate.
The figures in parentheses indicate the number of rats in the experiments.

Acid-ether extraction.— This procedure was carried out with the object of purification and isolation of the factor or factors. In the experiment by Jukes[3] and Wolley *et al.*,[4] it is stated that pantothenic acid is extracted from the acidified aqueous solution of the liver filtrate with ether. Hoffer and Reichstein[6], and Subbarow and Hitchings[7] have demonstrated that the fraction extracted with acid-ether is in all probability pantothenic acid and is a component of the rat "filtrate factor." Furthermore, it is indicated that pantothenic acid is very labile to alkali.

In our studies on this subject, the following procedure was undertaken.— Charcoal eluate (=50 cc of fraction A) was dissolved in an equal volume of water, adjusted to pH 2.5 with H_2SO_4 and extracted continuously with ether for 72 hours. The ether was changed and the extraction continued for further 90 hours. Tested on rats, it was observed that the first ether extract contained about one-half of the activity present in the starting material, the second a very slight amount, and the residue about one-half.

A portion of the first fraction of the acid-ether extract was evaporated under

reduced pressure, and was dissolved in 5% NaOH. The mixture was heated on water-bath for 2 hours, cooled and neutralized with HCl. There was no appreciable activity in the material thus treated when fed at the level equal to 20 g (or more) fresh liver (Fig. 5). It was, therefore, assumed that pantothenic acid was destroyed in this treatment.

The acid-ether residue was also heated in the same manner as above in the presence of 5% NaOH. The neutralized substance showed slight growth activity when given to rats (Fig. 6).

Acetylation.— A portion (=50 cc of fraction A) of the filtrate, after precipitation with mercury acetate, was evaporated to dryness under reduced pressure and taken up in a mixture of 15 cc of pyridine and 65 cc of acetic unhydride. The solution was allowed to stand at room temperature for several days. The reaction mixture was evaporated in vacuo and the residue was nearly soluble in chloroform (the insoluble residue appeared to be inactive). Into the chloroform solution an equal volume of water was added and shaken, and the chloroform layer was separated from the aqueous layer.

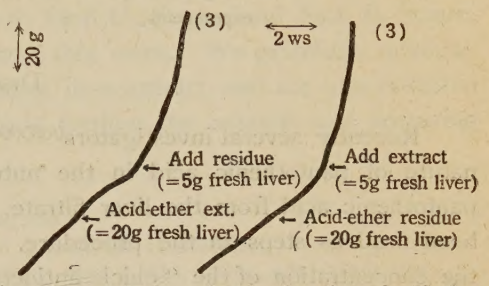

Fig. 5. Growth curves showing the effects of acid-ether extract and residue supplements.
The figures in parentheses indicate the number of rats in the experiments.

Chloroform layer.— This was evaporated and hydrolized with $N/10$ sodium methoxide, standing at room temperature overnight, and then neutralized with HCl. This was concentrated under reduced pressure and prepared for assay.

Aqueous layer.— This was evaporated and hydrolized with $N/10$ sodium methoxide as above and tested on rats.

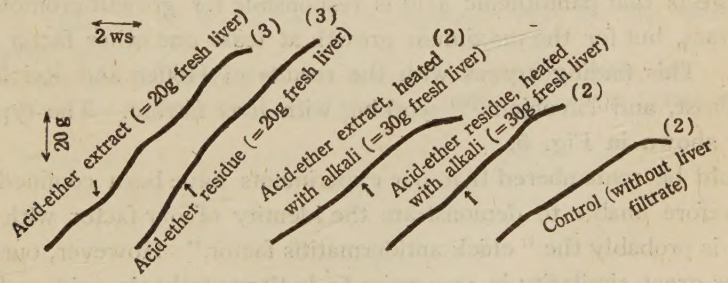

Fig. 6. Growth curves showing the effect of acid-ether extract and residue, and of both fractions heated with alkali.
The figures in parentheses indicate the number of rats in the experiments.

The hydrolized fractions of both chloroform and aqueous layers stimulated the growth of rats as seen in Fig. 7, while the unhydrolized fraction produced only a very slight response.

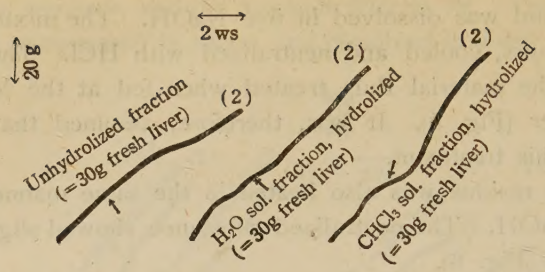

Fig. 7. Growth curves of rats on control ration, supplemented with acetate and its hydrolized fractions.
The figures in parentheses indicate the number of rats in the experiments.

Discussion.

Recently several investigators[6][7][8][10] have reported evidences for the essential nature of pantothenic acid in the nutrition of the rat. In the concentration of pantothenic acid from the liver filtrate, acetylation and acid-ether extraction have been used as steps in the procedure. Wooley *et al.*[9] have used this method in the concentration of the "chick antidermatitis factor" or pantothenic acid. Hoffer and Reichstein,[6] and Subbarow and Hitchings[7] have evidence indicating that pantothenic acid is the active component of their ether extract. Lunde and Kringstad[11] have stated that factor W is not extractable with acid-ether and that this factor can be differentiated from pantothenic acid.

We have separated two fractions by acid-ether extraction and believe that the factor in the ether extract is probably pantothenic acid and the factor in the residue is factor W of Frost and Elvehjem.[2] The growth rate was significantly greater when both fractions were given together than when each fraction was administered alone. This supplementary effect of the acid-ether extract and the residue suggests that pantothenic acid is responsible for growth-promoting function of liver extract, but for the maximum growth at least one other factor (factor W ?) is required. This finding agrees with the results of Hoffer and Reichstein,[6] and of Black, Frost, and Elvehjem,[12] working with liver filtrate. The typical growth response is shown in Fig. 5.

It should be remembered that our experiments have been confined to rats and we are therefore unable to demonstrate the identity of our factor with pantothenic acid, which is probably the "chick antidermatitis factor." However, our liver filtrate factor shows great similarity in properties to both pantothenic acid and the growth factor termed factor W. Final proof as to the relationship of these factors must await further study.

Summary.

1. Procedures are described for the concentration of the rat filtrate factor complex.

2. This complex is not adsorbed by acid clay; it is, however, adsorbed by large amount of charcoal, from which the active substances are eluted with 90% ethanol containing 0.1% pyridine.

3. The factor (or factors) is not precipitated by either phosphotungstic acid or mercury acetate.

4. It is not inactivated by acetylation; mild hydrolysis of this material produces a good growth response, the unhydrolized substance, however, possesses only slight activity.

5. The supplementary effect of the acid-ether extract and residue suggests that in addition to pantothenic acid, the rat requires an additional factor (factor W ?) for the normal growth.

We wish to express our deep gratitude to Prof. U. SUZUKI and Prof. B. SUZUKI for their advice and encouragement throughout this work. We gratefully acknowledge Dr. F. INUKAI's gift of a large amount of liver extract and are also indebted to Miss Sizuye OTSUKA for her willing help in feeding the animals and preparing the materials.

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ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

On the Formation of *l*-Ethylene-Oxide-α,β-Dicarboxylic Acid by Moulds. Part V.

(pp. 1015～1016)

By Kin-ichiro SAKAGUCHI and Tatuitiro INOUE.

(Agricultural Chemical Laboratory, Tokyo Imperial University; Received October 23, 1940.)

Acetic and *dl*-lactic acids were identified among the metabolic products from glucose by *Monilia formosa*[1] beside *l*-ethylene-oxide α-,β-dicarboxylic, citric and succinic acids which were already reported to be formed[2]. Fenton's reaction[3] which is characteristic of tartaric acid, was also given by an ether insoluble residue, but the isolation of the acid responsible for the reaction was not accomplished.

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On Lactose-fermenting Yeast.

(pp. 1017～1037)

By Motokiti HONGŌ.

(Agricultural Chemical Laboratory, Tokyo Imperial University; Received September 19, 1940.)

Über Nutzbarmachung des Vitamins C aus dem Pflanzenreich in Taiwan (IV. Mitteilung).

Über die Reaktionen zwischen Ascorbinsäure und MgO.

(SS. 1038～1040)

Von Ryo YAMATO und Takeshi HARA.

(Agrikulturchemisches Laboratorium, Taihoku Kaiserliche Universität, Taiwan;
Eingegangen am 9. 10. 1940.)

Wir haben festgestellt, dass reine Ascorbinsäure in wässriger Lösung mit MgO im Molekulargewichtsverhältnis 1 : 0.5 zu dem Salz geführt werden kann, welches den Mg-Gehalt von 6.69 % zeigt (als $(C_6H_7O_6)_2Mg$ 6.49 %), $[\alpha]_D^{19} = +96.5°$; und im Molekulargewichtsverhältnis 1 : 40 oder mehr zu einem unlös-

lichen Verbindungskörper geführt und von diesem Körper unter Zusatz von Säure wieder zu einer Lösung zurückgeführt werden kann. Von dieser Lösung isolierten wir die Krystalle der Ascorbinsäure.

Biochemical Studies on a Nutritional Yeast Preparation.

(pp. 1041～1044)

By Tetutaro TADOKORO and Naomoto TAKASUGI.

(Department of Science, Hokkaido Imperial University; Received October 3, 1940.)

On the Cellullose Analysis and Bleaching Methods of Cellulose Materials. Part III.

Modified Method for New Cellulose Estimation by Bleaching Powder.

(pp. 1045～1056)

By Sin-iti HONDA.

(Kyoto Imperial University; Received October 19, 1940.)

In the previous papers the present author had modified Jenkins and Norman's cellulose estimation method with bleaching powder[1] and as to the original method, they reported that the bleaching powder procedure was more advantageous and excellent than the NaOCl procedure. These results were also shown in the previous paper.

The present author tried to omit the so-called neutral treatments with NaOCl and 3% Na_2SO_3. This idea is conformable with the experimental results of Norman and Shrikhande[2], that hemicellulose as well as cellulose combined with lignin, and the neutral treatments with NaOCl and Na_2SO_3 was not effective for elimination of hemicellulose. In the present paper, it is shown that the elimination of neutral treatments gave no serious effects for the analytical purpose as shown in Table I. Thus the procedures of the analyses were much simplified.

Table I. Comparison of analysis by various methods with bakkoyanagi (*Salix Caprea L*). (Oven dry state.)

Method of chlorination. / Procedure of Analysis. / Component	Gasous state.	Liquid state (State of solution.)			
	Modified procedure of Cross & Bevan's Method.	NaOCl-Method (Jenkins & Norman's)		Bleaching Powder Method (author's method)	
		Previous procedure	Improved procedure	Previous procedure	Improved procedure
Total cellulose (%)	54.95	47.68±0.66	53.51±0.41	55.69±0.32	57.26±0.66
α-cellulose (ash-free) (%)	37.27	39.88±0.16	39.39±0.26	39.26±0.41	39.55±0.41

In total cellulose ;—					
α-cellulose (%)	67.83	83.59±1.02	73.39±0.16	70.60±0.31	69.86±0.30
α-cellulose ash (%)	—	0.36	0 14	0.09	0.19
β-cellulose (%)	32.16	16.05	17.22	28.45	29.95
γ-cellu'ose (%)	—	—	9.03	—	—
Number of chlorination. (1)	?	2N, 7A.	5A.	2N, 3A.	3A.

(1) Notations are according to Jenkins and Norman.

The total cellulose content given by the modified method was increased by about 1.5 %, for example, 57.26 % instead of 55.69 % by Jenkins' method.

Such differences were considered to be due to the incomplete separation of hemicellulose from the total cellulose fraction, but for the practical purpose, especially for the paper pulp analysis, it may be sufficient.

The α-cellulose contents, which is important for rayon pulp analysis, considered with Jenkins' original method and thus the α-cellulose analysis, owing to the simplicity of the analytical process.

In the previous paper, comparisons of using 2 % Na_2SO_3 instead of 3 % in Jenkins' original method in every Na_2SO_3 treatment were given.

In the present paper, the writer applied 2 % Na_2SO_3 treatments, thus the difficulty of 3 % Na_2SO_3 solution treatment was avoided.

The results are shown in Table II.

Table II. Extraction of sodium sulphite solution by boiling with yūlin-sun (*Picea ajanensis Fisch.*). (Oven dry state.)

After 1 gram of sample refluxed with 2 % Na_2SO_3 10 minutes, filtered and dried, then analyzed the components.

	Original wood. (%)	Residue of treatment (based on the original wood.) (%)	Extracted contents by 2 % Na_2SO_3 solution. (%)
	A	B	(A—B)
Moisture	10.26±0.16	—	—
Extracted contents by Na_2SO_3.	—	6.28±0.01	6.28
Lignin (1)	28.81±0.13	27.76±0.05	1.05
Methoxyl in lignin.	16.78±0.40	15.25±0.05	—
Pentosan	12.09±0.09	11.48±0.10	0.61

(1) Lignin was estimated by the following procedure; Samples extracted with alcohol-benzene (1:1) mixture and hot water, hydrolyzed with 72 % H_2SO_4 in ice chest for 48 hours, then diluted to 3 % and refluxed 3 hours.

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(Prof. Sikata's Laboratry, The Institute of Chemical Research, Kioto Teikoku-Daigaku)

Researches on the Electrolytic Reduction Potentials of Organic Compounds. Part XXVIII.

The Quantitative Analyses of Sugars by the Polarographic Method. (1). The fundamental experiments for the analyses of pentoses and pentosan.

(pp. 1057～1063)

By Isamu Tachi.

(Agricultural Chemical Institute, Kyoto Imperial University;
Received October 2, 1940)

Pentoses and pentosan are able to be quantitatively determined by the polarographic method by means of the estimation of furfural derived from them. The author has investigated the relation between the concentration and the height of the reduction wave of furfural, because this is very important for the polorographic analysis. It was shown that if the height of wave was measured by the so-called tangent point method the relation was shown with a straight line which passed through the original point of the co-ordinates. It was revealed that furfural was quantitatively produced from xylose, which was taken as a sample of pentoses, when xylose was heated with sp. g. 1.060 HCl for 2～3 hours at 160°C.

Pentosan in a hard wood which was produced in Siam was measured by the polarographic method and the value agreed with the value obtained by the phloroglucide method.

On the Application of Hydrogen Peroxide for Brewing. (Part IX.)

On the Catalase of Aspergillus oryzae.

(pp. 1064～1070)

By Hisao Matui.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;
Received September 20, 1940.)

The properties of the catalase excreted by kozi fungi (Aspergillus oryzae) were studied in culture medium and the results are summarized as follows:—

1. When kozi fungi are cultivated in kozi extract, the catalase action of the medium reaches the maximum in 7～10 days culture and then gradually decreases.
2. The inactivation of kozi-catalase first appeared when heated above 60°, suddenly becoming emphasized above 75°.
3. Kozi-catalase reacts with the monomolecular reaction at 1°, but at a higher temperature the velocity constant falls with the lapse of time.
4. When the temperature of the reaction mixture (0.008 N H_2O_2) is above 30°, the catalase is inactivated strikingly by H_2O_2 in it.
5. The optimum temperature for the catalase action is 30° or a little higher.
6. The optimum pH for the catalase action is about 7.

7. It has been observed that the natural salt (NaCl) added to culture medium (kozi extract) influences the formation of the catalase of A. oryzae; i. e., with the addition of NaCl in 2～5% the enzyme formation decreases, while in 7～10% it is increased.

8. If the kozi fungi are cultured on different substrata, these latter influence the catalase formation.

Strains of A. oryz.	A	B	Strains of A. oryz.	A	B
Kozi extract	1.3*	81.0	Henneberg's medium	9.5	61.0
Pfeffer's medium	57.3	4.6			

(Figures show the activity of the catalase in 30 days cultures)

9. The catalase is more effectively extracted from kozi (A. oryzae grown on steamed rice) with 0.25～0.5 % salt solution than with pure water, but if the salt content is over 0.5 %, the elution of the enzyme suddenly decreases.

10. When the catalase is extracted from kozi, the higher (up to 55°) the temperature, the more the enzyme is extracted.

11. The Taka-diastase includes the catalase of kozi fungus, and the optimum hydrogen ion concentration for this enzyme is about 7.*

12. The diastase preparation prescribed by the Japan pharmacopoeia (malt diastase) contains little catalase.

On the Application of Hydrogen Peroxide for Brewing. Part X.

On the Catalase of Moulds and Yeasts.

(pp. 1071～1073)

By Hisao Matui.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;
Received September 20, 1940.)

The catalase of thirty-seven strains of moulds—Aspergillus (19), Monascus (1), Penicillium (5), Rhizopus (6), Mucor (4) & Absidia (2)—and five strains of yeast —Saccharomyces saké, S. cerevisiae, S. ellipsoideus, Willia & Torula sanguinea— was investigated.

1. Of all the moulds A. oryzae excretes a particularly large amount of catalase when it is cultured on kozi extract, and A. flavus and A. melleus rank next.

2. There is a relation between the catalase action of the culture medium and the age of culture; i. e., when the moulds like A. oryzae, which excrete comparatively large amount of enzyme, are cultured the catalase action of the culture medium reaches the maximum in 1～2 weeks and then declines gradually, while when the moulds which excrete a small quantity of enzyme are cultured,

the catalase action still increases little by little even after cultivation for 30 days.

3. Although yeasts also excrete catalase in the culture medium, the amount of the enzyme is far less than that of A. oryzae.

On the Fatty Oil of Awa (Setarica itarica, Beauv) Bran.

(pp. 1074~1076)

By Yoshikatsu Mano.

(The Institute of Scientific Research, Manchoukuo; Received October 22, 1940.)

Some of the values of the fatty oil were estimated.

Also the fatty acids of this oil were classified approximately as follows:—

Total fatty acids { Solid fatty acids .....about 10.6%
Liquid fatty acids.....about 89.4%

..... { Fatty acids of oleic acid series ...about 10.6%
Fatty acids of linolic acid series ...about 80.7%

The unsaturated fatty acids were converted to their respective oxy-fatty acids and from the properties of these latter the identity of each original fatty acid was deduced.

On Xylitol. (I)

Preparation of Xylitol by Catalytic Reduction with Hydrogen under Pressure and the Uses of Xylitol.

(pp. 1077~1079)

By Teijiro Yabuta and Kiyoshi Aso.

(Agricultural Chemical Laboratory, Tokyo Imperial University;
Received September 30, 1940.)

Exchangeable Calcium and Magnesium of Soils in Tyosen.

(pp. 1080~1088)

By Hideo Misu.

(Agricultural Experiments Station, Government General of Tyōsen; Received June 6, 1940.)

Untersuchungen über Vitamin in Obstsaftfabrikaten. (II).

Einfluss des Unterschiedes der Klärung und der Lagerung auf den Vitamin B_1-Gehalt in Apfelsinensaft.

(SS. 1089～1097)

Von Tyoten Inagaki und Susumu Ohashi.

(Lebensmittelchemisches Forschungsinstitut der Meiji Zuckerindustrie;
Eingegangen am 19. 9. 1940.)

Es wurden Untersuchungen ausgeführt über den Vitamin B_1-Gehalt mit 4 Apfelsinensaftbüchsen und 1 Apfelwein von inländischen Waren.

Nach der *p*-Aminoacetophenon-Methode mit dem Pulfrichphotometer kommt im frischen Apfelsinensaft ein Vitamin B_1-Gehalt von durchschnittlich 10.7 γ auf je 100 g.

Durch wiederholte Untersuchungen wurde die Abwesenheit von gebundenem Vitamin B_1 im frischen Apfelsinensaft bestätigt.

Weiter wurde die enzymatische Klärung von Apfelsinensaft unter besonderer Berücksichtigung der Filtrationsenzyme untersucht, sowie die Klärung von Apfelsinensaft reich an Vitamin B_1 und die beste Lagerungsmethode zur Aufbewahrung.

On the Vitamin Contents of Dried Mushrooms Produced in Manchoukuo.

(pp. 1098～1100)

By Hideo Miyayoshi and Kozo Kawakami.

(The Institute of Scientific Research, Manchoukuo; Received October 22, 1940.)

Certain vitamin and ergosterol contents of *Pleurotus seratinus* (Schrod) Fr., *Armillaria mella* (Vahl) Fr., etc, were estimated and the following results obtained:—

	$B_1\gamma$ per 100 g	$B_2\gamma$ per 100 g	$C\gamma$ per 100 g	Ergosterol gr.%
Pleurotus seratinus	33.4	1292.7	25.312	0.250
Armillaria mella	8.0	52.5	11.237	0.300
Cortinellus Shiitake	—	526.0	17.777	0.277

On the Denaturation of Sericin. (Part 2.)

Isoelectric Point of α-sericin.

(pp. 1101～1106)

By Zirô Hirose.

(Sericultural Research Laboratory of Gunze Raw Silk Mfg. Co. Ltd;
Received October 12, 1940.)

(1) Introduction.

In the previous paper, we studied denaturation of sericin retained in the raw cocoon layers, caused by boiling in hot water; and found denatured sericin (retained sericin) in cocoon layers took up more anionic chromiate complex and minor cathionic chromiate complex than the original one, corresponding to the time of treatment. But in this treatment, 5～10 % of sericin was extracted from the raw cocoon layers. So we can easily imagine, that this difference of tanning capacities between retained (insoluble) and extracted (soluble) sericin fractions may be due to the modification of physico-chemical structures between both cases, and to the denaturation of sericin occurring in the process of cocoon boiling, and that isoelectric point of α-sericin in soluble and insoluble sericin fractions may have different encies accordingtend to the modification of their ionic structures.

In this paper we studied isoelectric point of α-sericin in soluble and insoluble sericin fractions. But in this and further reports, we mean α-sericin by one which can be obtained as precipitate when making pH of sericin sol 3.2～5.2, and β-sericin by one which can be obtained as precipitate from the filtrate of α-sericin by increasing the concentration of alcohol up to 50 %, adding ethanol to the filtrate.

(2) Experimental.

(A). The modification of isoelectric point of α-sericin in soluble and insoluble sericin fractions.

When sericin is extracted from the same raw cocoon layers by treating with boiling water for a short time repeatedly, water in each case being renewed, it is clear that soluble sericin fraction is extracted at the very beginning, and corresponding to the time of extraction, from soluble to insoluble fractions are being extracted. In this part we studied isoelectric point of α-sericin in soluble and insoluble fractions obtained according to the above idea. The procedure was as follows;—

45 gr. of raw cocoons, carefully freed from chrysalid, were extracted by boiling for 10 minutes in 3 *l.* of distilled water. The extraction was repeated 4 times, water in each case being renewed. The nitrogen contents in each extract was determined and compared with that of filtrate which was obtained by filtering off the precipitate caused by addition of the acetate mixture (final conc. – 0.02 m) of various hydrogen ion concentration. The difference of the two values gives the amount of sericin precipitated, and the pH value where the highest precipitate was

formed was taken as the isoelectric point of α-sericin. Experimental result was as follows;

(Quantity of N is expressed in mg/200 cc.)

Number of Extractions	Total Nitrogen	Kind of Sericins \ pH	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
1	29.12*	α-Sericin N.	14.56	14.91	15.33	18.62	18.76	18.94	19.88 (max)	17.15
		β-Sericin N.	14.56	14.21	13.79	10.50	10.36	10.18	9.24	11.97
2	14.21*	α-Sericin N.	9.59	9.66	9.67	9.98	10.17	10.22 (max)	9.87	8.19
		β-Sericin N.	4.90	4.83	4.82	4.51	4.32	4.27	4.62	6.30
3	6.09*	α-Sericin N.	3.39	3.39	4.68	4.87 (max)	4.27	3.39	3.37	3.35
		β-Sericin N.	2.70	2.70	1.41	1.22	1.82	2.70	2.72	2.74
4	5.11*	α-Sericin N.	—	3.57	3.71 (max)	3.29	8.22	3.01	—	—
		β-Sericin N.	—	1.54	1.40	1.82	1.89	2.10	—	—

The table clearly shows that isoelectric point of α-sericin in the first extract, or the most soluble sericin fraction, is more on the alkaline side than others, corresponding to their solubility. But the questions arise from this fact in these two points,

1. This fact may be due to the denaturation of sericin during the process of extraction.

2. Modification of isoelectric point of α-sericin slightly depends upon the concentration* of sericin sol[3] (See Literature).

So to verify this fact, the following experiments were carried out.

1. Influence of heating aqueous sericin sol on the modification of isoelectric point of α-sericin.

The aqueouse extract at 100°C. for 10 minutes, which contained 16.94 mg.N /200 cc., of which 12.88 mg. belongs to the α-sericin at pH 4.4 (isoelectric point), was boiled for 30 minutes under the reflex condenser, and experiment was carried out in the same way as described above.

(Quantity of N is expressed in mg/200 cc).

Kind of Sericin Sol.	Total Nitrogen	Kind of Sericin \ pH	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
Sericin Sol Boiled.	16.94	α-Sericin N.	2.24	3.50	3.99	3.99	5.11	5.16	5.46 (max)	5.39
		β-Sericin N.	14.70	13.44	12.95	12.95	11.83	11.78	11.48	11.55
Control.	16.94	α-Sericin N.	—	—	9.73	9.52	10.71	12.74	12.88 (max)	12.81
		β-Sericin N.	—	—	7.57	7.42	6.23	4.20	4.06	4.13

The difference was not found about the isoelectric point of α-sericin.

2. The modification of isoelectric point of α-sericin in soluble and insoluble sericin fractions with consideration of their concentration.

The extraction method was the same as described above, but water in each case was diminished corresponding to the times of extraction to obtain the approximately same sericin concentration of each extract.

(Quantity of N is expressed in mg/200 cc).

Number of Extractions.	Total Nitrogen	Kind of Sericin \ pH	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8
1	17.36	α-Sericin N.	—	—	4.97	5.88	6.51	6.85 (max)	6.02	5.04
		β-Sericin N.	—	—	12.39	11.48	10.85	10.50	11.34	12.32
2	15.40	α-Sericin N.	—	—	10.50	10.92	12.74 (max)	10.99	10.99	10.64
		β-Sericin N.	—	—	4.90	4.48	2.66	4.41	4.76	—
3*	16.94	α-Sericin N.	10 15	10.36	11.13 (max)	10.85	10.71	10.57	—	—
		β-Sericin N.	6.16	5.95	5.18	5.46	5.60	5.74	—	—

* 3rd and 4th extracts were collected into one.

Through these experimental results, it is clear that isoelectric point of α-sericin in soluble sericin fraction is more on the alkaline side than that of the insoluble one, corresponding to their solubility.

(B). Modification of isoelectric point of α-sericin between the outside and the inside layer of raw cocoon.

Regarding the difference of tanning capacities and difference of the solubility of the sericin in outside and inside layer of raw cocoon, we reported in the previous paper, together with the reason for these facts.

In this part, we studied modification of isoelectric point of α-sericin which was obtained by boiling outside and inside layer of raw cocoon respectively with distilled water for only 10 minutes.

1. In the case of α-sericin in outside layer.

25 gs. of outside layer was extracted by boiling for 10 minutes in 4 *l.* of distilled water.

(Quantity of N is expressed in mg/200 cc).

Total Nitrogen.	Kind of Sericin \ pH	4.0	4.2	4.4	4.6	4.8
17.93	α-Sericin N.	8.93	9.21	9.91	10.89 (max)	9.14
	β-Sericin N.	9.00	8.72	8.72	7.02	8.79

2. In the case of α-sericin in inside layer.

32 gs. of inside layer was extracted by boiling for 10 minutes in 2 *l*. of distilled water.

(Quantity of N is expressed in mg/200 cc).

Total Nitrogen.	pH / Kind of Sericin	3.4	3.6	3.8	4.0	4.2	4.4	4.6
19.67	α-Sericin N.	9.17	9.45	9.94	10.15	10.50	11.06 (max)	10.71
	β Sericin N.	10.50	10.22	9.73	9.52	9.17	8 61	8.96

These two tables clearly show that isoelectric point of α-sericin in outside layer of raw cocoon is more alkaline than that in the inside layer, corresponding to their solubility.

3. Summary.

The work included in this paper may properly be summed up as follows;—

(1) Isoelectric point of α-sericin in soluble sericin fraction is more alkaline than insoluble one, corresponding to their solubility.

(2) Isoelectric point of α-sericin in outside layer of raw cocoon (soluble sericin) fraction is more alkaline than that in inside layer (insoluble sericin fraction) confirming the above result [Summary (1)].

4. Literature.

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Untersuchung über die Beziehungen von Bataten zur Alkoholproduktion.

(SS. 1107~1129)

Von Y. Takeda, M. Suematu. und M. Utikosi.

(The Institute of Research on Chemical Industry, Government-General of Taiwan;
Received 21. 9. 1940.)

Studien über die Flavingärung der Aceton-Butylalkoholbakterien. I.

(SS. 1130～1140)

Von Izue YAMASAKI.

(Aus dem Agrikulturchemischen Institut der Kaiserlichen Kyusyu-Universität in Hukuoka; Eingegangen am 14. 10. 1940.)

Relation Between Oil Content of Fish Liver and Vitamin A Content of Liver Oil.

(pp. 1141～1150)

By Hideo HIGASHI.

(Imperial Fisheries Experimental Station, Tokyo, Japan;
Received September 28, 1940.)

The vitamin A content of fish liver oil is influenced by various factors, e. g., age, sex, spawning, fishing season, fishing ground and oil content of liver, etc. If the other factors are nearly equal, the vitamin A content of liver oil has direct connection with the oil content of liver, i. e., vitamin A content of liver oil becomes very rich when the oil content of liver decreases. This fact is observed about almost all species of fish.

The author's results are as follows.

Species	Fishing Ground	Fishing Season	Sex	Body Length (cm)	Body Wt (g)	Liver Wt (g)	Oil Content of Liver (%)	C.L.O.U.
Katsuonus vagan (L.).	Adjacent Sea of Palau	April, 1936	Female	64.0	6800	56.0	5.16	122
				64.0	7100	61.0	3 53	244
Sebastodes flammeus J. and S.	Off the Coast of Shiogama	May 29th. 1936	Male	33.0	1056	23.1	26.8	204
				33.0	1125	19.8	18 7	325
Sebastodes flammeus J. and S.	Off the Coast of Shiogama	Jan. 28th. 1937	Male	36.0	1230	27	25.4	170
				36.0	1190	19	13.8	487
				36.0	1070	21	9.3	720
Sebastodes flammeus J. and S.	Off the Coast of Shiogama	Feb. 27th. 1937	Male	34.0	1098	20	14.5	242
				34.0	1108	24	9.7	440
Sebastodes iracundus J. and S.	Off the Coast of Mito	May 6th. 1936	Female	57.3	5800	126	46.2	130
				57.8	5275	95	22 7	568
Sebastodes iracundus J. and S.	Off the Coast of Choshi	May 13th. 1936	Female	50.0	3938	63	32.8	130
				50.0	3638	53	21.0	975
Sebastodes iracundus J. and S.	Off the Coast of Shiogama	May 29th. 1936	Female	53.0	4500	93	16.2	975
				53.0	4300	67	14 7	920

Sebastodes iracundus J. and S.	Off the Coast of Shiogama	May 29th. 1936	Male	50.0	3700	45	16.0	650
				50.0	3575	74	14.1	720
				50.0	3000	45	11.3	1450
Sabastodes iracundus J. and S.	Off the Coast of Shiogama	Jan. 29th. 1937	Female	47.0	2320	28	22.5	348
				47.0	2610	30	13.7	1462
Sebastodes matsubarae (H.).	Off the Coast of Mito	May 29th. 1936	Female	43.0	2000	27	15.8	568
				43.0	2000	25	11.5	1140
				43.0	2000	27	10.6	1210
Sebastodes matsubarae (H.).	Off the Coast of Shiogama	Sep. 3rd. 1936	Female	45.0	2600	62	30.1	146
				45.0	2600	41	22.2	975
Brama raii (B.).	Off the Coast of Katsuura	April 13th. 1939	Female	35.0	950	11.5	4.21	120
				35.0	810	9.0	4.06	150
				35.0	945	8.5	3.71	210
Seriola quinqueradiata T. and S.	Off the Coast of Nagasaki	Sep. 20th. 1938	Male	60.0	4265	35	13.3	42
				60.0	3855	30	6.5	210
				60.0	3775	20	2.95	490
Seriola quinqueradiata T. and S.	Off the Coast of Nagasaki	Sep. 20th. 1938	Male	63.0	4245	42	5.35	60
				63.0	4030	41	1.92	336

In *Sebastodes flammeus* J. and S. and *Sebastodes iracundus* J. and S., oil content of liver (F) and vitamin A content of liver oil (C. L. O. U.) (A) have been determined for many individuals. According to these results the relation between F and A can be expressed as follows:

$$\log F = b - a \log A \quad \text{(I)}$$

or

$$a' - F = b' \log A \quad \text{(II)}$$

where a, b, a' and b' are constants.

Equation (I) is proposed by the author, and equation (II) has been proposed by Schmidt-Nielsen. The former is more applicable to the case of *Sebastodes flammeus*, but the latter to the case of *Sebastodes iracundus*. In the case of *Theragra chalcogramma* (P.), either equation (I) or (II) holds good. Consequently both equations are applicable to many species of fish, but in some species equation (I) holds more true and in others equation (II).

On the Retting of Vegetable Fibre Materials. Part XIV.

(pp. 1151~1156)

By Hideo KATAGIRI and Tosio NAKAHAMA.

(Department of Agriculture, Kyoto Imperial University; Received October 14, 1940.)

In the previous papers, it was proposed by us that a useful retting bacteria revealed effective action only upon a certain kind of vegetable fibre materials.

In order to get further evidence for these specificities of retting bacteria, pectin decomposing enzymes of these bacteria were compared.

All the useful retting bacteria including one species of bacteria for ramie, four species for hemp, three species for flax, two species for kenaf and one species for jute fibre materials, were found to reveal very much the same activity of pectase with which Ca-tartrate was produced from methyl-*d*-Ca-tartrate.

The action of pectinase with which lemon pectin was decomposed, was found to be different amon gthe species of retting bacteria, i. e. *B. linumus* for flax, *Achromobacter venosum* for flax, *Microc. cannabis* for hemp, and *Listerella hibiscus liquefaciens* for kenaf attacked pectin very remarkably, while *B. subtilis* for ramie, *B. cannabis* for hemp and *Kurthia cannabis liquefaciens* for hemp attacked slightly on pectin.

Therefore, any parallel relation was not found to exist between the kinds of fibre materials and the activity of pectase or pectinase of the bacteria.

However, very remarkable specificities were pointed out between the activity of bacterial protopectinases and the kinds of protopectin prepared from various kinds of fibre materials.

These specificities of bacterial protopectinases were found to be very much the same as those of the bacterial rettings of vegetable fibre materials.

Biochemistry of Bakanae Fungus. Part VII.

The Cultural Condition for Producing Gibberellin or Fusaric Acid. II.

(pp. 1157~1158)

By T. Yabuta, Y. Sumiki, E. Katayama and H. Motoyama.

(Agricultural Chemical Laboratory, Tokyo Imperial University;
Received October 25, 1940.)